REMARKS/ARGUMENTS

Claims 20-21, 24, 26, 28-31, and 35-38 are pending. No amendment is made to any of the pending claims. Reconsideration of the present application in view of the following remarks is respectfully solicited.

Applicants have gratefully acknowledged that in view of Applicants' previously submitted persuasive arguments the Examiner has now withdrawn the rejection of claims 20-21, 24, 26, and 28-31 under 35 U.S.C.§ 112, first paragraph, as not enabled.

However, the Examiner issues several new grounds of rejection. Applicants respectfully traverse.

I. Obviousness rejection of claims 20-21, 24, 26, 28-31,35-36, and 38

The Examiner rejects claims 20-21, 24, 26, 28-31,35-36, and 38 as being unpatentable under 35 U.S.C. § 103(a) over Roth et al (U.S. Patent No. 5,545,535) in view of Akai et al. (U.S. Patent No. 5,891,731) and Yue ST (U.S. Patent No. 5,656,449). We disagree.

The present application is directed to a method of preparing an assay sample for detecting bacteria by a flow cytometer. The method comprises:

providing a **diluent** comprising a cationic surfactant, a **buffer for maintaining a pH of 2.0-4.5** and an effective amount of **a substance capable of reducing nitrite ions** and a staining solution comprising a polymethine dye for staining bacteria;

mixing a urine sample with the diluent; and

preparing the assay sample by mixing the mixture of the urine sample and the diluent with the staining solution. .. . *See*, e.g., claim 20.

Another independent claims claim 38, which is directed to a method of staining bacteria, comprises the same steps as recited in claim 20. The remaining pending claims all depend from claim 20.

The newly cited primary reference Roth discloses a method of analyzing a sample for bacteria. The method comprises using an aqueous solution comprising one or more fluorescent dyes. At col. 16, lines 45-52, Roth discloses that the aqueous solution is made by dissolving the dyes directly in water or a buffer or in an organic water-miscible solvent, such as DMSO, DMF, methanol, or ethanol. Without citing any supportive evidence, the Examiner states that DMSO is a well-known nitrite ion reducing agent. Therefore, the Examiner concludes that Roth teaches all of the limitations of the pending claims except for the features concerning: 1) cationic surfactant; 2) mercaptoethanol, and 3) the pH of 2.0 to 4.5. The Examiner then relies upon other secondary references, including Akai and Yue, to remedy the deficiencies associated with Roth.

For reasons set forth below, the cited art, taken together, fails to provide any apparent reason that a person of ordinary skill in the art would have **combined "the known elements in the fashion claimed by the patent** at issue." *See KSR International Co. v. Teleflex Inc. (KSR),* 550 U.S.398, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007); MPEP2141 (The key to supporting any rejection under 35 U.S.C. 103 is the clear articulation of the reason(s) why the claimed invention would have been obvious.)

First, none of the cited art discloses a step of providing a **diluent** as recited in the claims of the present application or **a step of mixing a urine sample with the diluent prior to the step of mixing the urine sample with a staining solution.** Note that it is clear from the claims of the present application that the diluent and staining solution are two different things. The diluent is mixed with the urine sample first, and then the staining solution is mixed with the diluent and urine sample. Assuming that the Examiner is correct that the DMSO, which is used as a solvent in Roth, is a well-known nitrite ion reducing agent, then Roth at most discloses a staining solution comprising a dye and DMSO as the solvent. Nor does any cited art discloses a step of mixing a diluent as recited in the claims of the present application before the staining solution is mixed with the urine sample. Indeed, none of the cited art provides any reason to do so. Unlike

the present application, none of the cited art recognizes the benefit of reducing the nitrite ions in the urine sample to improve the accuracy of the fluorescence assay.

Similarly, regarding the use of mercaptoethanol, Yue discloses that beta-mercaptoethanol may be used in a **staining solution** for greater storage stability. Yue does not disclose that beta-mercaptoethanol should be used in a diluent, which is separate from an aqueous solution until a sample is mixed with the aqueous solution and then assayed, as a nitrite reducing agent.

Second, none of the cited art discloses a diluent comprising a buffer for maintaining a **pH of 2.0-4.5.** Rather, the cited art teaches away from the pH range recited in the claims of the present application. For example, Roth discloses several specific pH values, including 7.4, 10, and 8.2 (*see* col. 10, Table 1, footnotes 1 and 4, col. 36, line 39), which all depart significantly from the range recited in the claims of the present application. Akai discloses at col. 8, lines 23-37 that the suitable pH range is 6.0-11.0, preferably 7.0-11.0, and more preferably 8.0-9.5. According to Akai, "when the pH is lower than this range, erythocytes become fragile and hemolysis is apt to take place whereby accurate measurement of reticulocytes becomes difficult." Yue discloses at col. 6, lines 14, that the pH of the staining solution is typically between 6.5 and 8, which is significantly different from the range recited in the claims of the present application.

Moreover, Applicants do not believe that the Examiner correctly applies the rule of "optimization from general conditions" to conclude that the pH range recited in the claims is merely routine optimization. As stated in MPEP2144.05.II.A, optimization can only be done within prior art conditions or through routine experimentation. Here, as noted above, none of the cited art discloses a general pH range, which would cover or at least be close to, the range recited in the claims of the present application. Nor does any of the cited art disclose any reason to use a much lower range as recited in the claims of the present application.

Third, the unexpected results of the present application further show that the present application is not obvious in view of the prior art. The specification of the present application discusses and shows extensively various benefits of using a nitrite reducing agent and a lower pH range. *See*, for example, pages 18-20, Examples 1-2 and page 15, lines 1-5. None of the cited art provides any reasonable expectation of these benefits.

Therefore, claims 20-21, 24, 26, 28-31,35-36, and 38 are not obvious over Roth in view of Akai and Yue under 35 U.S.C. § 103. Withdrawal of the rejection of these claims over Roth in view of Akai and Yue under 35 U.S.C. § 103 is respectfully requested.

II. Obviousness rejection of claim 37

Claim 37 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Roth in view of Akai and Yue, as applied above to claims 20-21, 24, 26, 28-31,35-36, and 38, and further in view of Inoue (U.S. Patent No. 5,891,733).

The Examiner only relies upon Inoue's disclosure concerning the use of ethylene glycol. Inoue cannot remedy any deficiency as discussed above in connection with other cited art. Therefore, for at least the same reasons discussed above in connection with claims 20-21, 24, 26, 28-31,35-36, and 38, claim 37 is also not obvious over Roth in view of Akai, Yue, and Inoue under 35 U.S.C. § 103(a).

It is noted that like other cited art, Inoue also discloses at col. 6, lines 52-55 that the optimal dyeing pH value is at pH 5.0 to 9.0, preferably at pH 6.5 to 7.5. This further supports Applicants' statement, as noted above, that the cited art teaches away from the pH recited in the claims of the present application.

Therefore, claims 37 is not obvious over Roth in view of Akai and Yue, and further in view of Inoue under 35 U.S.C. § 103. Withdrawal of the rejection of these claims over Roth in view of Akai and Yue, and further in view of Inoue under 35 U.S.C. § 103 is respectfully requested.

III. Double Patenting Rejection

Claims 20-21, 26 and 38 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-4 and 8 of Sysmex's own U.S. Patent No. 7,422,870 issued to Kawashima et al. in view of Yue discussed above. We disagree.

Neither Kawashima nor Yue discloses a step of providing a **diluent** as recited in the claims of the present application or **a step of mixing a urine sample with the diluent prior to the step of mixing the urine sample with a staining solution**. As noted above, Yue discloses that beta-mercaptoethanol may be used in a **staining solution** for greater storage stability. But Yue does not disclose that beta-mercaptoethanol should be used in a diluent, which is separate from an aqueous solution until a sample is mixed with the aqueous solution and then assayed, as a nitrite reducing agent.

Therefore, the cited art, taken together, fails to provide any apparent reason that a person of ordinary skill in the art would have **combined "the known elements in the fashion claimed by the patent** at issue." *See KSR, supra*, 550 U.S.398.

Accordingly, the nonstatutory obviousness-type double patenting rejection of claims 20-21, 26 and 38 over claims 1-4 and 8 of Kawashima et al. in view of Yue should be withdrawn.

Based on the foregoing, Applicants believe that the present application is now in condition of allowance. Early and favorable consideration is earnestly requested.

If any additional fees or charges are required at this time, they may be charged to our Patent and Trademark Office Deposit Account No. 03-2412.

Respectfully submitted,
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